Cancer Genetics Solutions

Agilent
Trusted Answers

Pushing the Boundaries in Cancer Genetics

Cancer is a formidable foe that presents significant challenges. The complexity of this disease can be daunting due to the number of mechanisms that can trigger carcinogenesis, including the influence of environmental factors. However, each new discovery can reveal new understanding that can get us one step closer to unraveling this complexity.

Innovation drives discovery

Leaders in cancer genetics research drive innovations to address complexity and provide a better understanding of targets that may show promise for conquering this disease. At Agilent we continually develop innovative, multi-omic solutions to help you overcome the hurdles of cancer genetics research.

Accelerating the pace

Providing complementary, advanced molecular solutions for cancer genetic analysis is a core mission of Agilent. Our unparalleled leadership in the development and customization of technologies enables you to focus on the most important targets, increase the pace of discovery, and accelerate breakthroughs.

| The Range of Genetic Aberrations Detected by Precision Genetic Technologies							
Technology	Point mutation	Indel	Gene copy number change	Balanced translocation	Structural variant	LOH	Gene regulation
DNA Seq Target Enrichment	√	√	√	√		√	
RNA-Seq		√	√	√	√		√
Methyl-Seq							√
Gene Expression Microarrays							√
CGH+SNP Microarrays			√		√	√	
FISH			√	√	√		
qPCR	√	√	√				

Analysis of Structural & Single Base Changes

Translocations, loss of heterozygosity (LOH) and copy number variations (CNVs) can all influence susceptibility to cancer[1], and single nucleotide polymorphisms (SNPs) are useful biomarkers to identify genomic regions associated with cancer.

SureSelect Target Enrichment products for next-generation sequencing (NGS) provide the best tools for sensitive, accurate and comprehensive identification of these somatic variants in both solid tumors and hematological samples. Catalog and custom options provide superior performance with either exome captures or targeted panels.

Design your own custom SureSelect DNA panels using intuitive SureDesign software, and your own custom SureSelect RNA panels using intuitive eArray software.

– Detect LOH, CNVs, and mutations associated with SNPs using low input DNA amounts

– Obtain high genome-wide NGS coverage and uniformity

– Choose from catalog and custom target enrichment probes

– Complete your target enrichment step in as little as one day

Deep Coverage with FFPE Tumor Samples
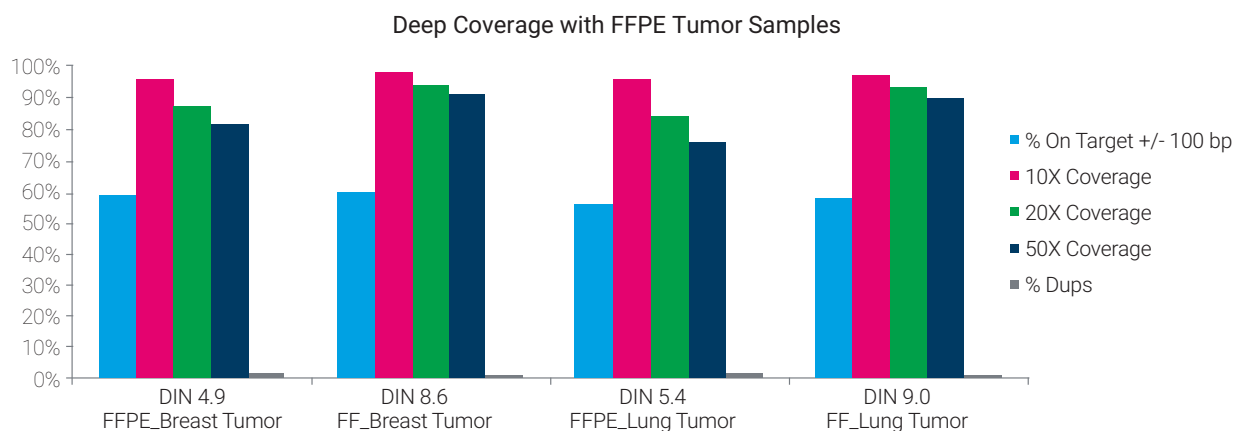
Figure 1. Sequencing performance of SureSelect[XT] libraries from FFPE and fresh-frozen (FF) breast and lung tumor samples enriched using the ClearSeq Comprehensive Cancer research panel (240 Mb sequencing/sample). The level of DNA degradation is indicated by the DNA Integrity Number (DIN) provided by the 2200 TapeStation System, where DINs of 10 and 1 indicate gDNA and completely degraded DNA, respectively.

SureSelect Catalog Designs Optimized for Cancer Research	
Product	**Features**
SureSelect Human All Exon V6+COSMIC	Comprehensive exome-wide analysis, including COSMIC targets
SureSelect Human All Exon V6 +UTR	Comprehensive transcriptome (RNA) and exome (DNA) analysis
ClearSeq Comprehensive Cancer	Enables analysis of 151 key genes associated with a wide range of cancers
ClearSeq DNA and RNA Kinome	Analysis of the kinome, including UTRs

# Detection of Rare Genetic Variants

Cancer can be caused by rare, low-penetrance genetic variants ranging from allele frequencies of 0.1% or less up to almost 5%[2], and extraordinary genetic heterogeneity occurs in both solid and hematologic cancers[3].

Agilent HaloPlex[HS] Target Enrichment products enable sensitive and accurate detection of rare variants in cancer samples. HaloPlex[HS] is a combination of amplicon-based and target enrichment sequencing that delivers accurate results across many genes and from very low DNA inputs. Molecular barcodes enable identification and elimination of sequencer and PCR errors in high coverage NGS data.

"The HaloPlex[HS] technology using molecular barcodes gave us the highest ratio of accuracy and sensitivity to identify low frequency mutations in all the samples."

– Ioannis Ragoussis,
Ph.D. McGill University

– Confidently detect mutations below 1% allele frequency in genetically heterogeneous samples

– Differentiate true variants from PCR or FFPE artifacts by targeting both DNA strands

– Get high on-target specificity and deep coverage of target bases so key variants are not missed

– Perform target enrichment in <6 hours from only 50 ng of gDNA

Detection Down to 0.5% Allele Frequency
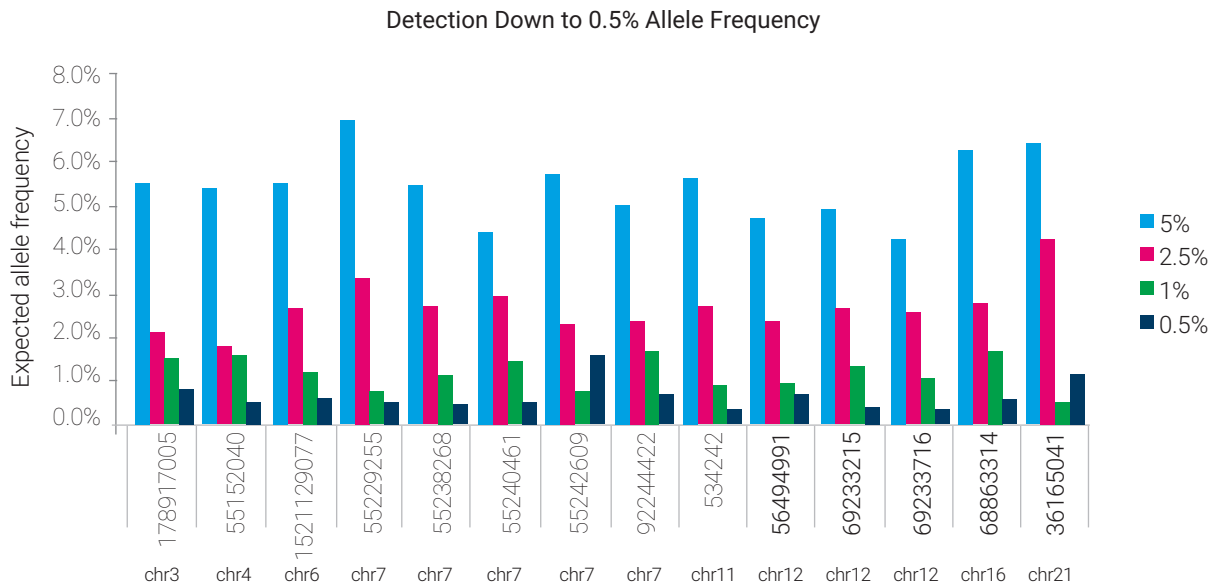
Figure 2. HapMap cell lines, NA18507 and NA10831, were mixed to generate allelic fractions ranging from 0.5%-5%. The close agreement between expected and observed frequency at various chromosomal positions demonstrates the high sensitivity of HaloPlex[HS] for low frequency variant detection. Data shown is representative of replicates (sequencing depth=2000x-4000x)

Gene Expression and Gene Regulation in Cancer

RNA splicing disruption is common during cancer genesis[4], resulting in significant variations in gene expression. Gene expression changes can lead to unregulated cell division and cancer.

The SureSelect Strand Specific RNA Library Preparation Kit is the ideal discovery tool to find novel splice variants, non-coding elements, antisense transcripts and gene fusions by NGS, with the highest sensitivity for preparing libraries for mRNA or targeted RNA-Seq.

SurePrint G3 Gene Expression Microarrays provide 5-log dynamic range and produce data that correlates better to RNA-Seq experiments than competitor arrays.

- Generate more efficient captures of the top 1,000 expressed genes with RNA-Seq
- Use the lowest 5′ and 3′ bias target enrichment chemistry in the industry
- Employ a strand-specific RNA-Seq approach to find and confirm antisense transcripts[5]
- Target difficult fusions, like BCR-ABL, where shorter baits may fail to hybridize to the full transcript

Low Duplication Rate
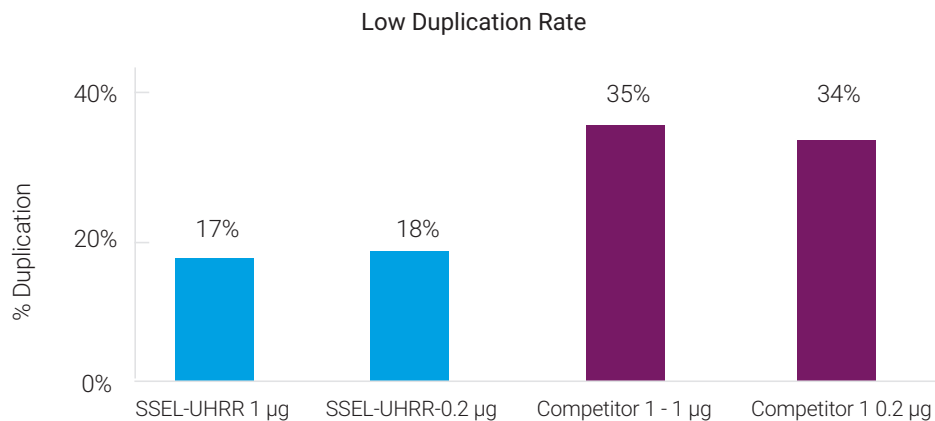
Figure 3. SSEL Strand Specific Library Prep for RNA had a duplication rate below 20% whereas the competitive platform had a duplication rate above 30% under the same conditions. In this experiment, all reads were normalized to 20 million/library (2X100 bp sequencing) for comparison. Universal Human Reference RNA was the input sample for both platforms to provide equivalence. Each platform was tested with 1 µg and 200 ng. Before library prep, Poly A purification was performed for all samples.

Copy Number Variation is a Key Driver in Cancer Genesis

A larger percentage of the genome in cancers is affected by somatic cell copy number variants (CNVs) than any other kind of somatic genetic alteration[6].

They play important roles by inactivating tumor suppressors and activating oncogenes, and the study of CNVs has driven substantial advances in cancer diagnostics and therapeutics. Analysis and characterization of CNVs in hematological cancers have improved significantly through the use of array comparative genomic hybridization (aCGH)[7]. Agilent catalog and custom CGH microarrays provide exceptional sensitivity and flexibility with the most challenging sample types.

The quality of Agilent CGH technology allows faster and more efficient association of disease research phenotypes with genotypes.

The GenetiSure Cancer Research CGH+SNP Array combines exon-level resolution with the detection of copy number and copy-neutral changes.

- Detect aberrations even in heterogeneous samples due to high signal-to-noise ratios, and report clonal fraction
- Process samples faster than other microarray methods
- Select from a comprehensive menu of products, including labeling solutions for fresh, frozen and FFPE samples
- Design genome-wide custom arrays free of charge with no minimum order

Accurately Detect Low Level Deletions
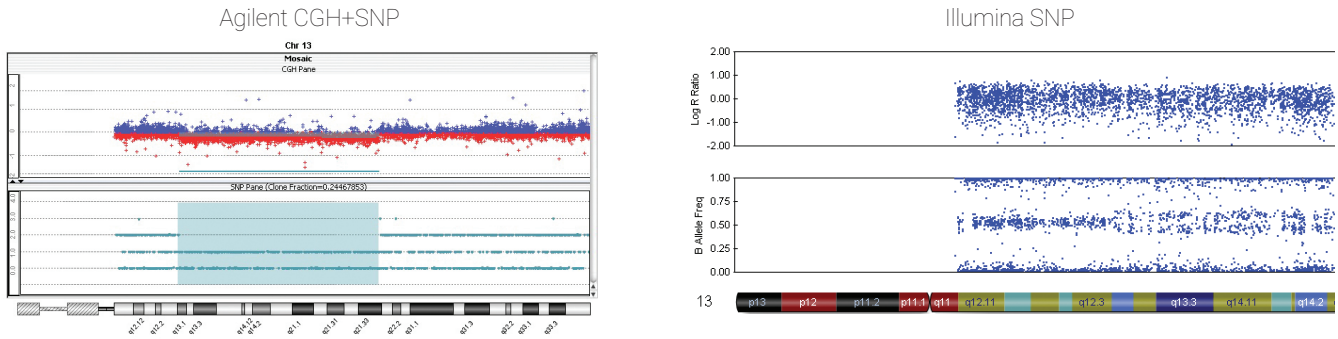
Figure 4. DNA CN and SNP profile of chromosome 13 of a Multiple Myeloma sample hybridized on Agilent SurePrint G3 CGH+SNP 2×400K microarrays and Illumina SNP microarrays analyzed with the respective vendor software. Results indicated that the deletions present in approximately 25% of the cells could be detected on Agilent's platform in both the CN and SNP data, but was only detectable as an aberration in the Illumina SNP data.

The Importance of Sample Quality

Many factors can impact quality and/or quantity of the DNA and RNA extracted from samples, including cold ischemia, fixation (FFPE) and processing.

An accurate understanding of starting sample quality is absolutely required, as it can significantly affect the quality of genomic and transcriptomic profiling. The Agilent 4200 TapeStation and 2100 Bioanalyzer systems provide automated electrophoresis for DNA and RNA sample quality analysis for any NGS, microarray, or quantitative PCR workflow. Automation frees up researcher time and reduces errors, while providing both DNA (DIN) and RNA (RIN) integrity numbers.

– Get fully automated sample processing for up to 96 samples with the 4200 TapeStation System

– Maximize assay flexibility with on-chip electrophoresis of DNA or RNA or proteins with the Agilent 2100 Bioanalyzer System

– Run the NGS FFPE QC Kit on the AriaMx fully integrated quantitative PCR system to enable assessment of the integrity of DNA as well as accurate quantitation of amplifiable template
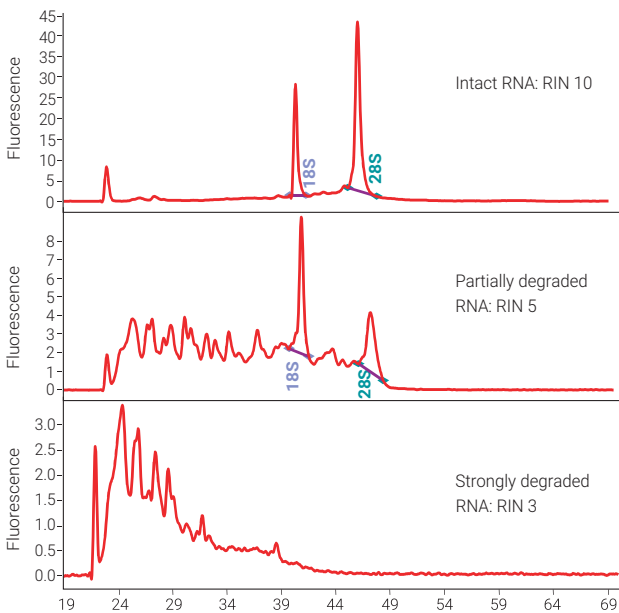
Figure 5. 2100 Bioanalyzer System: The established RNA Integrity Number (RIN) provides quality assessment of total RNA.
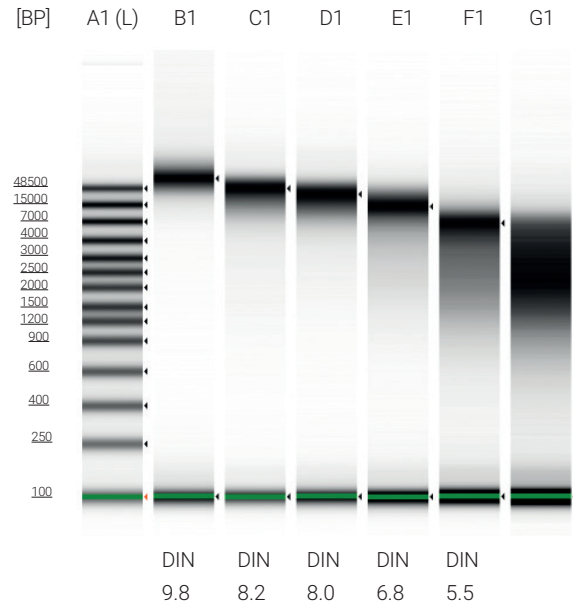
Figure 6. 4200 TapeStation System: Genomic DNA samples at different degradation stages with DNA Integrity number (DIN).

References

1. Bente *et al.* Genetic variation and its role in malignancy. Int J Biomed Sci. (2011) 7: 158-171.

2. Bodmer W. and Tomlinson I. Rare genetic variants and the risk of cancer. Curr Opin Genet Dev (2010) 3: 262-267.

3. Spencer *et al.* Performance of common analysis methods for detecting low-frequency single nucleotide variants in targeted next-generation sequence data. J Mol Diagn. (2014) 16: 75-88.

4. Sveen *et al.* Transcriptome instability as a molecular pan-cancer characteristic of carcinomas. BMC Genomics (2014) 1471-2164-15-672.

5. Levin *et al.* Comprehensive comparative analysis of strand-specific RNA sequencing methods. Nature Methods (2010) 7: 709−715.

6. Zack *et al.* Pan-cancer patterns of somatic copy number alteration. Nat Genet. (2013) 45: 1134-1140.

7. Hartmann *et al.* Detection of clonal evolution in hematopoietic malignancies by combining comparative genomic hybridization and single nucleotide polymorphism arrays. Clin Chem (2014) 60: 1558-1568.

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